Complete Amino Acid Sequence of the Precursor Region of Rat Prolactin†

David J. McKean* and Richard A. Maurer

ABSTRACT: Rat preprolactin was translated in a wheat germ in vitro synthetic system in the presence of ^{3}H-labeled amino acids. The amino-terminal sequence of the radiolabeled precursor was quantitatively identified by high pressure liquid chromatography. The 29 residue precursor sequence is: Met-Asn-Ser-Gln-Val-Ser-Ala-Arg-Lys-Ala-Gly-Thr-Leu-Leu-Leu-Leu-Met-Met-Ser-Asn-Leu-Leu-Phe-Cys-Gln-Asn-Val-Gln-Thr-. A discussion of the structure–function relationships of the precursor region and the microsequencing methods used to determine the sequence is presented. The amino-terminal 30 residue sequence of the secreted form of rat prolactin was determined and found to differ at positions 7 and 8 (-Gly-Asp-) from a previously reported rat prolactin sequence (Parlow, A. F., & Shome, B. (1976) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 219).

Rat prolactin, like many other secretory proteins, is translated as a larger precursor form from mRNA in vitro (Maurer et al., 1976; Swan & Leder, 1972; Kemper et al., 1974; Boime et al., 1975; Suchanek et al., 1975; Devillers-Thiery et al., 1975; Sussman et al., 1976; Beato & Nieto, 1976; Jilka & Pestka, 1977; Strauss et al., 1977; Palmiter et al., 1977; Chan et al., 1976). In addition, the synthesis of the prolactin precursor (preprolactin) has been demonstrated in intact cells (Maurer & McKean, 1978). Previous studies have shown preprolactin to be of higher molecular weight than the secreted form (Maurer et al., 1976). This increased molecular weight of the precursor is due to an additional 29 amino acids on the amino terminus of prolactin (Maurer et al., 1977). Although the function of precursor regions is unknown, it has been hypothesized (Milstein et al., 1972; Blobel & Doberstein, 1975) that they act as a signal to orient the nascent peptide into the endoplasmic reticulum membrane in order to facilitate movement across the membrane and into the intracisternal space of the endoplasmic reticulum.

An amino acid sequence analysis of the 29 residue precursor region of rat preprolactin was undertaken in order to provide additional information for understanding the structure–function relationships of the precursor regions. In this paper we report the complete amino acid sequence of the 29 residue precursor to rat prolactin. Quantitative microsequencing methods were developed in order to complete this sequence which is the largest precursor yet completely identified. These techniques are presented and compared with other microsequencing methodologies. The amino-terminal sequence of the secreted form of rat prolactin was also determined and found to be different at two positions from the previously published sequence (Parlow & Shome, 1976).

Experimental Procedures

Materials. The preparation of rat pituitary mRNA, its translation in a wheat germ cell-free system and the isolation of the preprolactin were done as described previously (Maurer et al., 1976; 1977). Preprolactin was labeled with 15 amino acids in three separate reactions each containing five ^{3}H-labeled amino acids. The reaction mixtures (0.2 mL) included the following radioactive amino acids: (reaction 1) [2,3-^{3}H]-Asp[1] (15.8 Ci/mmol) at 30 μCi; [^{3}H]Val (32.6 Ci/mmol) at 60 μCi; [4,5-^{3}H]Leu (10 Ci/mmol) at 20 μCi; [3-^{3}H]Ala (36 Ci/mmol) at 75 μCi; [3-^{3}H]Arg (11 Ci/mmol) at 20 μCi; (reaction 2) [^{3}H]Gln (21 Ci/mmol) at 40 μCi; [3-^{3}H]Phe (25 Ci/mmol) at 50 μCi; [3,4-^{3}H]Pro (29 Ci/mmol) at 60 μCi; [3-^{3}H]His (11 Ci/mmol) at 20 μCi; [^{3}H]Trp (30 Ci/mmol) at 60 μCi; (reaction 3) [4,5-^{3}H]Ile (15.1 Ci/mmol) at 30 μCi; [^{3}H]Ser (8.9 Ci/mmol) at 20 μCi; [3-^{3}H]Glu (23 Ci/mmol) at 40 μCi; [4,5-^{3}H]Lys (25 Ci/mmol) at 50 μCi; [3,4-^{3}H]Tyr (41 Ci/mmol) at 80 μCi. All amino acids were purchased from Amersham Corp. except [^{3}H]Asp and [^{3}H]His which were purchased from New England Nuclear Corp. Each of the labeled amino acids was present at approximately 10 μM and unlabeled amino acids were present at a final concentration of 40 μM/reaction. After incubation the reaction mixtures were made 1% in Triton X-100 and 0.5% deoxycholate and were centrifuged at 10 000*g* for 10 min. Individual reaction mixtures were pooled and combined with 10 μg of unlabeled carrier rat prolactin. The mixture was then immunoprecipitated with rabbit anti-rat prolactin antisera. Immunoprecipitates were centrifuged through a discontinuous sucrose gradient, solubilized, and chromatographed on Sephadex G-25 (0.8 × 30 cm) in 0.05 M NH_4HCO_3 (Maurer et al., 1977). Preprolactins labeled with one ^{3}H- and one ^{14}C-labeled amino acid were similarly prepared. The purity of the cell-free translated preprolactin as determined by polyacrylamide gel electrophoresis has been previously reported (Maurer et al., 1977).

Sequencing Procedures. Each sample containing radiolabeled preprolactin, unlabeled carrier prolactin, and rabbit antibody was mixed with 1 mg of carrier egg lysozyme and 1 mg of dithiothreitol. The samples were sequenced with a Beckman Model 890C sequencer using a DMAA program (Vrana et al., 1978). Heptafluorobutyric acid cleavage was not included during the first sequencer cycle (cycle 0, Figure 3) in order to help remove any contaminating free amino acids

† From the Department of Immunology, Mayo Medical School, Rochester, Minnesota 55901, and the Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa. *Received June 23, 1978.* This work was supported in part by Research Grant CA22941 from the National Institutes of Health (to Dr. McKean), American Cancer Society Grant No. NP-254, and a grant from the Iowa State Cancer Registry (to Dr. Maurer).

[1] Abbreviations used: amino acids are abbreviated by either the three letter or single letter code (Dayhoff, 1972); Pth, phenylthiohydantoin; HPLC, high pressure liquid chromatography.

0006-2960/78/0417-5215$01.00/0

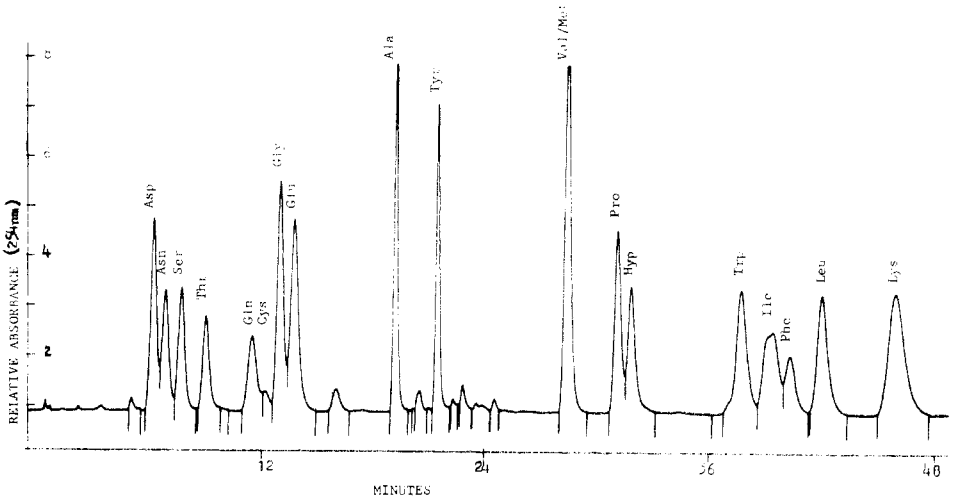

FIGURE 1: High pressure liquid chromatography of Pth-amino acid standard on a Du Pont Zorbax ODS column. Vertical lines before and after each peak indicate the electronic signal generated by the column monitor which moves the fraction collector to the next scintillation vial. Chromatographic conditions are outlined under Experimental Procedures.

and to increase initial coupling efficiency of the sequencer sample. Sequence samples from precursor proteins containing two labeled amino acids were processed by dissolving the dried thiazolinone fractions in scintillation fluid (3a70B Research Products) and counting in a liquid scintillation counter.

A complete set of phenylthiohydantoin (Pth) amino acids (10 nmol) which included Pth-hydroxyproline was added to the butyl chloride containing thiazolinone fractions from the multiple amino acid labeled precursor protein sequences. The samples were dried with N_2 and converted to phenylthiohydantoins with 1 M HCl (80 °C, 10 min). The labeled Pths were separated on a Du Pont Zorbax ODS column with a Waters Model 660 liquid chromatograph using a program modified from Zimmerman et al. (1977). A 21-min program run at room temperature using 14% acetonitrile to 30% acetonitrile in 0.01 M sodium acetate, pH 3.5, concave gradient (no. 10) was used to separate all organic soluble Pth residues except Met and Val which coeluted (Figure 1). The aqueous soluble Pth-His and -Arg were separated with a 10-min gradient (no. 6) of 8% acetonitrile to 40% acetonitrile in 0.01 M sodium acetate, pH 3.5.

The absorbance of the column eluent was monitored continuously by an ISCO Model UA-5 column monitor which in turn controlled a fraction collector (ISCO Model 32B) with delay and fraction totalizer circuits so that each Pth peak was collected in separate scintillation vials. Scintillation fluid (3a70B, Research Products) was added to each vial and the vials were counted in a liquid scintillation counter. The scintillation counter data were corrected for quenching and sample losses (relative to the Pth-hydroxyproline internal standard). The corrected scintillation counter data were subsequently analyzed according to procedures established for conventional amino acid analyzer sequencer data (Smithies et al., 1971).

Rat prolactin utilized for sequence studies was isolated as described by Ellis et al. (1969). The amino-terminal 29 residue sequence was identified by HPLC of the Pth-amino acids and by amino acid analysis of back-hydrolyzed samples (Smithies et al., 1971).

Results

The precursor region of rat preprolactin was previously determined to be a 29-residue addition on the amino terminus of the prolactin molecule (Maurer et al., 1977). The length of the precursor was established by aligning the Met, Leu, and Cys residues which occur in the amino-terminal sequence of prolactin.

In order to complete the precursor sequence, a single translation reaction incorporating 15 ^{3}H-labeled amino acids was prepared. The translation efficiency of this 15 labeled amino acid reaction was very poor. The poor translation efficiency may have been due to contaminants in the labeled amino acids. A considerable residue was found after the 15 labeled amino acids were pooled and dried prior to translation of the mRNA. Subsequently, three separate translation reactions were prepared including five different labeled amino acids each. The reaction products were pooled after translation, purified together, and sequenced. Two such translation/sequence experiments of preprolactin labeled with 15 ^{3}H-labeled amino acids were completed and both gave similar sequence results.

Five amino acids were not labeled in the 15 ^{3}H-labeled amino acid translation reaction because (1) they had been previously identified in the sequence (Met, Cys) (Maurer et al., 1977), (2) they were not available as ^{3}H-labeled amino acids (Asn), or (3) the specific activities of the available ^{3}H-labeled amino acids were low (Thr, Gly; see Discussion).

Additional translation/sequence experiments were done in order to identify the presence of the three residues which had not previously been identified (Asn, Thr, Gly) or to confirm the assignment of those residues which were not positively identified in the multiple labeled preparations. Preprolactin was labeled with [^{3}H]Gly/[^{14}C]Asn, [^{3}H]Arg/[^{14}C]Thr, [^{3}H]Ser/[^{14}C]Thr, [^{3}H]Val/[^{14}C]Pro, or [^{3}H]His/[^{14}C]Thr, and each double labeled sample was independently sequenced.

Sequence Yields. Although the repetitive yields of our precursor sequences were usually equal to conventional sequences (92–94%), the initial yield (cpm per residue theoretical) at cycle 1 varied from 3% to about 40%. This variation in yield correlated with the particular wheat germ preparation used and not with the mRNA preparation or with the sequencer efficiency. Some wheat germ preparations yielded translation products which consistently gave a poor initial sequence yield (data not shown). The preprolactin sequence studies were completed with a single wheat germ preparation which gave relatively good initial yields. The wheat germ preparations which gave poor yields were presumably blocking the free α-amino group of the protein but we were unable to

determine the nature of this presumed blocking reaction. Inclusion of [^{14}C]acetyl coenzyme A in wheat germ reactions did not result in significant radiolabel incorporation suggesting that the block was not due to N-acetylation of the amino-terminal Met.

Amino Acid Sequence Determination of Preprolactin. We have previously reported that the length of the precursor region of rat preprolactin was 29 residues long and contained Met at positions 1, 17, and 18, Leu at positions 13, 14, 15, 16, 21, and 22 and Cys at position 24 (Maurer et al., 1977). The complete amino acid sequence of the precursor of preprolactin was determined after identifying the ^{3}H label that coeluted with Pth standards on the HPLC. The precursor sequence is: Met-Asn-Ser-Gln-Val-Ser-Ala-Arg-Lys-Ala-Gly-Thr-Leu-Leu-Leu-Leu-Met-Met-Ser-Asn-Leu-Leu-Phe-Cys-Gln-Asn-Val-Gln-Thr- (Figure 3).

Preliminary experiments (data not shown) demonstrated that each labeled Pth residue that had a baseline separation on the HPLC chromatogram (Figure 1) could be quantitatively collected into a single scintillation vial. Radioactivity coeluting with Pth residues which did not have baseline separation on the HPLC did increase the background radioactivity in the adjacent peak (e.g., position 23, Pth-Ile is high because of spillover from the adjacent PTH-Phe on the HPLC chromatogram). This spillover was not significant enough to interfere with the interpretation of the sequence identification. The HPLC program used in these studies essentially separated all Pth residues except Met and Val which coeluted. The Met was not radiolabeled in the 15 labeled amino acid mixture because it had been previously identified in the precursor sequence (Maurer et al., 1977).

Sequence results from preprolactin labeled with some ^{3}H-labeled amino acids consistently show high ^{3}H backgrounds. The high ^{3}H background associated with [^{3}H]Leu-labeled precursors coelutes with Pth-Leu on the HPLC and decreases significantly only after 20–25 degradation cycles (data not shown). The reason for this high background remains unexplained. The Pth-Pro containing peak (Figure 3) had a ^{3}H background which was considerably higher and more variable than any other Pth residue. This is the region of the chromatogram where extraneous peaks are observed during conventional sequencing analyses and may be due to breakdown products of one or more amino acids. The absence of Pro in the precursor segment was confirmed by an independent translation/sequence analysis of [^{3}H]Val/[^{14}C]Pro preprolactin. Proline was found to occur at position 31 in the preprolactin sequence. Position 31 of the preprolactin sequence corresponds to position 2 of the prolactin sequence.

In these studies Pth-Ser, presumably in its dehydroserine derivative, was found to coelute with Pth-Ala. It was subsequently found that, when thiazolinone-containing fractions from the sequencer are dried down at room temperature rather than heated to 50 °C, 50–70% of the Pth-Ser residues coeluted with the Pth-Ser standard. Ser residues were identified at positions 3, 6, and 19 on a separate translation/sequence determination. The different Pth-Ala/Ser peak heights from the 15 labeled amino acid precursor sequence are consistent with Ser at residues 3, 6, and 19 and Ala at residues 7 and 10 (see Figure 3). Pth-Gln was identified as a mixture of Pth-Gln and Pth-Glu while Pth-Glu elutes as a single peak.

The sequence of [^{3}H]Arg/[^{14}C]Thr preprolactin had a high background of ^{3}H at cycle 1. An initial coupling/washing cycle (cycle 0) without heptafluorobutyric acid was not run for this sequence analysis. The resulting ^{3}H at cycle 1 probably was due to the presence of free [^{3}H]Arg in the sequencer sample. [^{3}H]Arg was not found at cycle 1 on either of the two 15 labeled amino acid preprolactin sequences (data not shown).

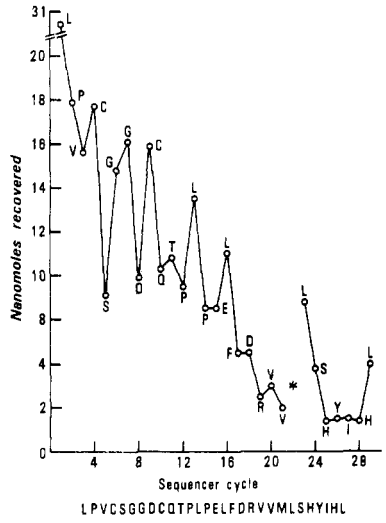

FIGURE 2: Amino-terminal sequence of rat prolactin. The sequence was quantitatively identified by amino acid analysis of the back hydrolyzed sequencer samples and corrected according to Smithies et al. (1971). The sequence identification was also analyzed by HPLC (data not shown). Residue 22 (*) is Met which was destroyed by acid hydrolysis but identified by HPLC. The prolactin amino-terminal sequence is summarized at the bottom of the figure.

During these studies the amino-terminal sequence of prolactin was determined in order to accurately align the sequence of the precursor region of preprolactin with that of prolactin. The amino-terminal 29 residue sequence of rat prolactin (Figure 2) differs at two positions, 7 and 8, from the sequence determined by Parlow & Shome (1976). This discrepancy may be due to genetic differences between rat populations or sequence errors. However, this cannot be resolved because a detailed account of the original prolactin sequence procedures and materials was not published.

Discussion

The 29-residue sequence of rat prolactin precursor is the longest precursor region yet completely identified. The additional molecular weight of the precursor appears to be due to these 29 extra amino acids at the amino terminus of the protein. Data from carboxypeptidase digestions of radiolabeled preprolactin are consistent with the precursor having the same carboxyl-terminal residue as the secreted form of prolactin (Maurer, unpublished results).

Sequence studies of rat preprolactin were originally initiated with the hope that an analysis of the precursor's primary structure would permit further understanding of the precursor's structure–function relationships. Like other precursors, the composition of the preprolactin precursor is hydrophobic. There is no obvious homologous primary sequence among the different precursors that could serve as a recognition site for a membrane receptor which might be common to the endoplasmic reticulum membrane of different cells. It may be that the hydrophobic nature of the precursor is sufficient to initiate transmembrane movement.

Amino acid sequence analyses of preprolactin samples which have been processed by rat pituitary cells or by dog pancreas membrane preparations have shown that the primary sequence of the precursor is cleaved between positions 29–30 (-Thr-Leu-) (Maurer & McKean, 1978). Other precursor regions are similarly specifically cleaved (Lingappa et al., 1977). Previous studies (Jackson & Blobel, 1977; Suchanek et al., 1978) have also shown that membrane preparations from

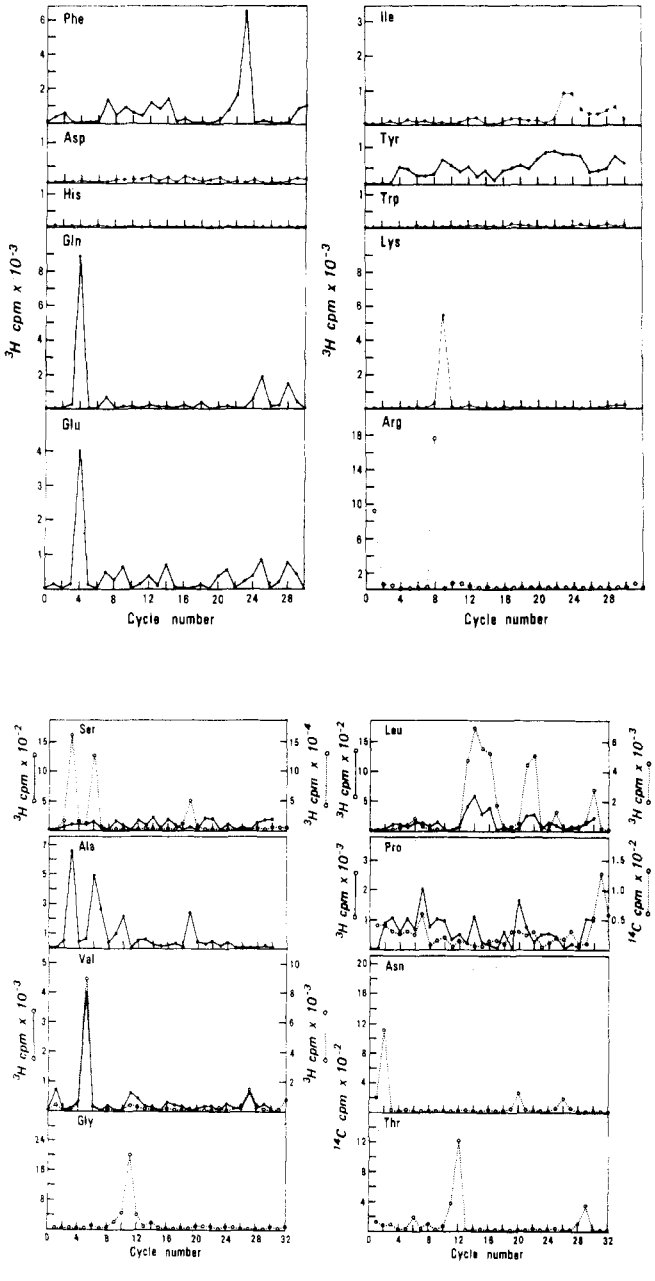

FIGURE 3: Analysis of the amino terminal sequence of rat preprolactin. Solid lines (● — ●) represent ^{3}H-labeled Pth-amino acids from the sequence of [^{3}H]preprolactin labeled with 15 ^{3}H-labeled amino acids and identified by HPLC analysis. Broken lines (○ - - - ○) represent radioactivity in the thiazolinone-containing fractions of each sequencer cycle from preprolactin labeled with one ^{14}C- and one ^{3}H-labeled amino acid. The amino-terminal sequence of rat preprolactin is:

```
                5                   10
Met-Asn-Ser-Gln-Val-Ser-Ala-Arg-Lys-Ala-Gly-

        15                  20                  25
Thr-Leu-Leu-Leu-Leu-Met-Met-Ser-Asn-Leu-Leu-Phe-Cys-Gln-

                                                30
                                    Asn-Val-Gln-Thr-Leu-
```

The Leu at residue 30 is the amino-terminal residue of rat prolactin (Maurer et al., 1977).

different tissues and different species are able to correctly process precursor proteins at specific peptide bonds. In spite of this apparent ubiquity and specificity of the processing enzyme(s), the nature of the enzymes' substrate specificity is not obvious. The carboxy-terminal residue of most precursor regions is not a single amino acid but rather a group of relatively neutral amino acids (Thr, Ser, Gly, Ala, Cys) (Strauss et al., 1977; Palmiter et al., 1977; Suchanek et al., 1978; Jilka & Pestka, 1977; Schechter et al., 1977). The amino-terminal residues of secretory proteins from which the precursor segment has been removed have an even greater diversity of amino acids (Leu, Ala, Asp, Glu, Pro, Arg, Lys, Val, Phe). Preprolactin has another -Thr-Leu- sequence (positions 12–13) within the precursor and yet we have never observed an amino-terminal sequence from partially processed preprolactin samples which would correspond to position 13 being the amino-terminal residue. Similarly, Ser, Gly, Ala, and Cys residues occur both in the precursor and the amino-terminal region of prolactin, and yet preprolactin is always processed between positions 29 and 30. The secondary or tertiary structure of the precursor segment may determine the point of cleavage by making the peptide bond between residues 29 and 30 accessible to the processing enzyme. Isolation of the processing enzyme(s) will probably be required in order to elucidate the substrate specificity of this enzyme(s).

Since the amounts of radioactivity incorporated in vivo or in vitro into proteins generally range from 10^2 to 10^4 cpm/residue, it is necessary to utilize quantitative analytical procedures in order to accurately identify the sequence of the radiolabeled protein. To determine the precursor sequence of preprolactin, we utilized commercially available equipment for the quantitative collection of radiolabeled Pth-amino acids from an HPLC. Chromatographic procedures are available for the complete separation of all 18 Pth organic-soluble residues with a single HPLC run (Zimmerman et al., 1977), although a given column chromatographic elution procedure is often not completely reproducible in different HPLC columns even from the same manufacturer.

Sequencing proteins by the analysis of a radiolabel presents difficulties not encountered with conventional amino acid sequencing techniques. Wide variations in the specific activities of the labeled amino acids in the radiolabeled protein can make an amino acid with high specific activity difficult to distinguish from a similar peak of radioactivity from a residue with low specific activity. Although a significant conversion of radiolabeled amino acids does not appear to occur in wheat germ cell-free systems (unpublished observations) as it does in intact cells (Ballou et al., 1976), specific activities of commercially available amino acids (especially ^{3}H amino acids) can vary by a factor of 50. Existing unlabeled amino acid pools in the wheat germ preparations make the specific activities of each amino acid in the radiolabeled precursor difficult to calculate. For example, although the specific activity of [^{3}H]Ala was greater than that of [^{3}H]Ser in the incorporation mixture, the relative peak heights of [^{3}H]Ser were greater than [^{3}H]Ala in the sequence data (Figure 3).

Determining sequences from data that has not been corrected for specific activities requires interpretation of plots of cpm/sequencer cycle for each individual amino acid (as in Figure 3). The sequence is determined by identifying the residue which has an increase in its signal to noise ratio at each sequencer cycle. If there is enough radioactivity in the sample to produce significant signal-to-noise ratios for each amino acid and if the sample contains a single, uniformly radiolabeled protein, the sequence determination is usually straightforward. However, when quantitating radiolabeled Pth residues by HPLC, unexplained increases in the background of individual Pth residues do occur (e.g., Pro at residues 7, 20; Ile at residue 24; Glu at residues 9, 14, and 31). If the amount of radioactivity in the sequencer sample is low, these spurious peaks could

be difficult to differentiate from the peaks generated by the sequence. Analysis of the radiolabeled Pth residues is also more difficult if not all the amino acids are accounted for in the sequence analysis or if two or more similar proteins (such as polymorphic membrane antigens) are being sequenced together. In spite of these limitations, the quantitative identification of a sequence from a radiolabeled protein permits proteins to be sequenced which would otherwise be impossible to detect by conventional sequencing techniques.

Acknowledgments

We would like to thank Mr. Michael Bell and Ms. Bobbi Maurer for their help in completing these experiments. We are also grateful to Ms. Theresa Lee for her assistance in preparing this manuscript.

References

Ballou, B., McKean, D. J., Freedlender, E. F., & Smithies, O. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 4487.

Beato, M., & Nieto, A. (1976) *Eur. J. Biochem. 64,* 15.

Blobel, G., & Dobberstein, B. (1975) *J. Cell Biol. 67,* 835.

Boime, I., Boguslawski, S., & Caine, J. (1975) *Biochem. Biophys. Res. Commun. 62,* 103.

Chan, S. J., Keim, P., & Steiner, D. F. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 1964.

Dayhoff, M. O. (1972) in *Atlas of Protein Sequence and Structure,* Vol. 5, National Biomedical Research Foundation, Washington, D.C.

Devillers-Thiery, A., Kindt, T., Scheele, G., & Blobel, G. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72,* 5016.

Ellis, S., Grindeland, R. E., Neunke, J. R., & Callahan, P. X. (1969) *Endocrinology 85,* 886.

Jackson, R. C., & Blobel, G. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74,* 5598.

Jilka, R. L., & Pestka, S. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74,* 5692.

Kemper, B., Habener, J. F., Mulligan, R. C., Potts, J. T., & Rich, A. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71,* 3731.

Lingappa, V. R., Devillers-Thiery, A., & Blobel, G. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74,* 2432.

Maurer, R. A., & McKean, D. J. (1978) *J. Biol. Chem.* (in press).

Maurer, R. A., Stone, R., & Gorski, J. (1976) *J. Biol. Chem. 251,* 2801.

Maurer, R. A., Gorski, J., & McKean, D. J. (1977) *Biochem. J. 161,* 189.

Milstein, C., Brownlee, G. G., Harrison, T. M., & Mathews, M. B. (1972) *Nature (London) New Biol. 239,* 117.

Palmiter, R. D., Gagnon, J., Ericsson, L. H., & Walsh, K. A. (1977) *J. Biol. Chem. 252,* 6386.

Parlow, A. F., & Shome, B. (1976) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 35,* 219.

Schechter, I., Burstein, Y., & Zemmel, R. (1977) *Immunol. Rev. 36,* 3.

Smithies, O., Gibson, D., Fanning, E. M., Goodfleish, R. M., Gilman, J. O., & Ballantyne, D. L. (1971) *Biochemistry 10,* 4912.

Strauss, A. W., Bennett, C. D., Donahue, A. M., Rodkey, J. A., & Alberts, A. W. (1977) *J. Biol. Chem. 252,* 6846.

Suchanek, G., Kindas-Mogge, I., Kreil, G., & Schreier, M. H. (1975) *Eur. J. Biochem. 60,* 309.

Suchanek, S., Kreil, G., & Hermodson, M. A. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75,* 701.

Sussman, P. M., Tushinski, R. J., & Bancroft, F. C. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 29.

Swan, D., Aviv, H., & Leder, P. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69,* 1967.

Vrana, M., Rudikoff, S., & Potter, M. (1978) *Proc. Natl. Acad. Sci. U.S.A.* (in press).

Zimmerman, C. L., Appella, E. A., & Pisano, J. J. (1977) *Anal. Biochem. 77,* 569.